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RESIDUAL INJURY CAUSED BY  
IRRADIATION WITH FAST NEUTRONS

by  
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**U.S. NAVAL RADIOLOGICAL  
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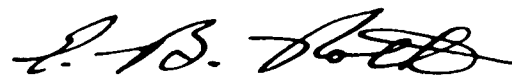
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ADMINISTRATIVE INFORMATION

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# ABSTRACT

The LD<sub>50</sub> of C<sub>3</sub>H female mice for fission-spectrum neutrons was found to be  $274.5 \pm 4.2$  rad. The previously reported\* value in the same mice for 250 kvp X-ray was 632 rad, giving an RBE of 2.30. Conditioning exposure of the mice to a total neutron dose of  $482.3 \pm 7.2$  rad over an interval of 8-10 weeks resulted in lowering the LD<sub>50</sub> for neutrons by  $41.8 \pm 5.9$  rad, or  $8.67 \pm 1.23$  percent of the total conditioning dose. The previously reported\* value in the same mice for 250 kvp X-ray was 9.58 percent of the conditioning dose. Continued exposure of the mice to 166 rad doses of neutrons at intervals of 4-5 weeks gave a median survival time of 22 weeks. This was greater than that produced by exposure to 420 rad and less than that produced by exposure to 280 rad of 250 kvp X-ray given at the same time. It is concluded that the RBE of the mice for residual radiation injury and the RBE for chronic radiation tolerance over short time intervals are the same as that for acute injury tolerance.

\* Radiation Research 10, 80-88 (1959).

## NONTECHNICAL SUMMARY

### The problem:

To determine whether fast neutrons have a greater relative biological effectiveness (RBE) for residual biological injury than for acute injury.

### The findings:

The residual injury was determined by measuring the amount by which a preliminary non-lethal exposure to radiation will reduce the acute LD<sub>50</sub> to the same radiation at a time when recovery from the preliminary exposure is complete. The residual injury caused by exposure of mice to fission-spectrum neutrons was found to be the same as that caused by exposure to X-ray (9-10% of the preliminary dose). On this basis, it was concluded that the RBE of neutrons for residual injury in mammals is the same as that for acute injury. A limited study of the tolerance of the mice to repeated irradiation to death indicated that the RBE for tolerance of repeated neutron irradiation was the same as that for tolerance of acutely lethal injury. A survey of the literature, however, indicates that the RBE for repeated neutron irradiation may increase if the irradiation lasts longer than 5 months.

## INTRODUCTION

The relationship between type of ionizing radiation and the degree of biological injury caused by it has usually been expressed as the relative biological effectiveness -RBE- defined as the ratio of the dose of a standard type of radiation to the dose of the tested type of radiation that gives an equivalent biological effect. A number of studies (1-4) have shown that for many short term biological responses in rats and mice (including acute lethality), irradiation with fast neutrons has an RBE of about 1.5 to 3.0, compared to X and  $\gamma$ -irradiation. There has further been an opinion that for delayed or residual radiation effects the RBE for neutrons as compared to X and  $\gamma$ -irradiation is much higher- perhaps as high as 13 or more (5). The principal support for this viewpoint is the evidence (6, 7) that animals exposed to low level chronic irradiation to death require much smaller rates of dose accumulation with neutron radiation than with  $\gamma$ -radiation for equivalent reduction in survival times. Because survival time under chronic exposure to radiation involves a number of interacting factors, among which accumulation of residual injury is only one, a study was made, and is herewith presented, of the actual amount of residual injury caused by exposure to neutron irradiation. The measurement of residual injury was made in terms of the non-

recuperable fraction of injury, using methods applied and reported previously for X-rays (8).

#### METHODS

Animals used in this study were  $C_3H$  female mice obtained from the Cancer Genetics Research Laboratory of the University of California at Berkeley. The experiments were begun when the animals were 10-14 weeks old.

Neutron irradiation was obtained from the 60 inch cyclotron of the Crocker Laboratory of the University of California by bombardment of a thick beryllium target with 12 Mev. protons. The neutrons produced had an energy distribution closely resembling that of the primary fission spectrum (9), with a flux at 20 inches from the target of  $3-5 \times 10^7$  neutrons/cm<sup>2</sup>/sec. and a dose rate of 20-50 rad/min. The animals were exposed by placing them in plastic tubes and attaching the tubes to a wheel which was rotated slowly in a plane perpendicular to the beam axis at a distance of 20 inches from the target (1).

X-radiation was obtained from a 250 kvp machine, 25 ma., HVL 2.3 mm/Cu, delivering 12-14 rad/min at 115 cm from the target. The mice were exposed by placing them in Lucite boxes, each consisting of three compartments measuring 20 x 8 x 8 cm, 4-5 mice per compartment, and arranging the boxes in a circular segment of the beam at a radial distance of 115 cm from the target.



Neutron dosimetry was performed by measuring the integrated neutron flux for each exposure with sulfur wafer threshold detectors, multiplying the flux by a conversion factor to give the calculated neutron dose in rads, then multiplying the calculated neutron dose by 1.07 to include the  $\gamma$ -ray contamination of the beam at the exposure distance used (9). X-ray doses were measured in air with a Victoreen thimble chamber r-meter. The X-ray air doses in r, were converted to tissue doses in rad by assuming that 1 r equals 93 ergs per gram of tissue.

The  $LD_{50}$  of mice to irradiation was determined by exposing separate groups of mice to serially increasing radiation doses, observing the 30-day mortality in each group, and calculating the regression of the probit of mortality on the logarithm of the dose, according to standard methods (10).

#### Experimental Design

The basic experimental design is shown in Figure 1. The principle of the experiment involves exposing a group of animals to a non-lethal series of radiation (the conditioning irradiation), allowing them to recover, and then measuring the 30-day  $LD_{50}$  of the animals for the same kind of radiation. The decrease in  $LD_{50}$  of the conditioning-irradiated animals, compared to controls, is a measure of the residual injury remaining from the conditioning irradiation, and is expressed as a fraction or percent of the total conditioning dose. This fraction is the constant " $\alpha$ " of the Blair formulation (11).

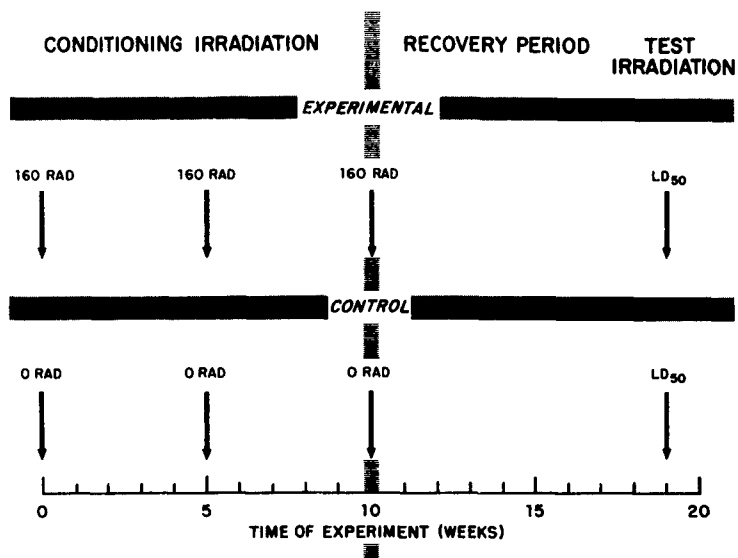


Fig. 1 Diagram of the experimental protocol for determination of non-recuperable injury.

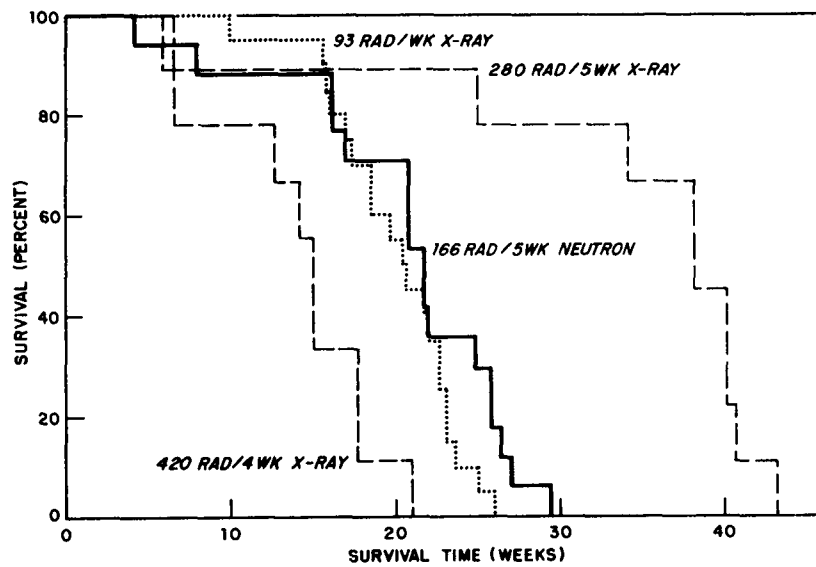


Fig. 2 Survival curves for animals given repeated X- or neutron radiation.

The conditioning exposure series shown in Figure 1 lists a group of 3 doses of 160 rad each at intervals of 5 weeks. In practice, both the size of the dose and the interval between exposures varied slightly, depending upon the performance of the cyclotron and beam time scheduling. Another experiment was also performed, where the procedure was identical to that illustrated in Figure 1, except that the recovery time between termination of conditioning radiation and test of  $LD_{50}$  was extended to 23 weeks instead of 9 weeks. Separate controls were used for the 9-week and 23-week recovery experiments, but as there was no significant difference between the control  $LD_{50}$ 's, the control  $LD_{50}$  was calculated from the combined results of both experiments.

For the determination of survival time under repeated radiation, a group of mice was exposed repeatedly to neutron doses of 166 rad at intervals of 4-5 weeks. Two other groups of mice, selected from the same pool of animals at the same time, were given X-ray exposures of 280 and 420 rad, respectively, between 1 and 5 days after each neutron exposure was given to the first group. The irradiation was continued until all animals were dead. The parameter used was the percentage of the original number of animals surviving as a function of the duration of the experiment.

## RESULTS

### A. Non-recuperable Injury Resulting from Neutron Irradiation.

The results of the experiments on non-recuperable injury after neutron irradiation are summarized in Table I. For mice given a total conditioning dose of 482 rad in 3 equal doses at intervals of 4-5 weeks, the acute LD<sub>50</sub> for neutron irradiation 65 days after completion of the conditioning irradiation was 42 rad lower than that of controls. In a similar experiment where the time between completion of the conditioning irradiation and running of the LD<sub>50</sub> test was 168 days, the LD<sub>50</sub> of the conditioning-irradiated animals was 54 rad lower than that of controls. In the latter experiment many of the animals were lost when the doses accidentally fell outside of the middlethal range because of inability to monitor the dose during exposure. Nevertheless, the two experiments are in basic agreement. The non-recuperable injury from the neutron irradiation was 8.67 and 10.83 percent of the conditioning dose for the two experiments, and the mean, weighted for the number of animals in each experiment, was 9.07 percent of the conditioning dose.

The value of non-recuperable injury obtained for neutron irradiation may be compared with that obtained for X-ray. In a previous publication (8) non-recuperable injury from X-ray in C<sub>3</sub>H female mice ranged between 6.4 and 10.6 percent of the conditioning dose, with a mean of 9.58 percent.

Table I

## Non-recuperable Injury After Fast Neutron Irradiation

Conditioning dose	Recovery time	<u>No. animals</u> <u>No. groups</u>	LD <sub>50</sub>	Non-recuperable injury	
(rad)	(days)		(rad)	(rad)	(%)
482.3	65	120/7	232.7	41.8	8.67
$\pm$ 7.2			$\pm$ 4.1	$\pm$ 5.9	$\pm$ 1.23
504.0	168	27/4	219.9	54.6	10.83
$\pm$ 35.0			$\pm$ 2.9	$\pm$ 5.1	$\pm$ 1.26
0 (controls)	---	181/9	274.5	---	---
			$\pm$ 4.2		

Table II

## Chronic Exposure to Neutron and X-ray Radiation

Type of radiation	No. of animals	Mean dose per exposure rad	Mean interval between exposures days	Mean dose rate rad/week
neutron	17	166	34	38.5
X	8	420	28.4	104
X	8	280	34.6	56.7
X	20	93	7.0	93

Thus, the percentage of non-recuperable injury produced by neutron irradiation is not significantly different from that produced by X-irradiation.

The mean survival time for animals dying from the neutron irradiation was 10.8 days, and this survival time was not significantly affected by presence or absence of prior conditioning irradiation. The number of animals dying 5 days or less after exposure to the irradiation was insignificant.

B. Relative Biological Effectiveness of Neutron Irradiation for Acute Lethality.

The value obtained in the current experiments for the  $LD_{50}$  of control mice (274.5 rad) can be compared with previously obtained values of  $LD_{50}$  for X-ray in the same strain of mice (632 rad) (8). The calculated RBE for neutron irradiation thus is 2.30. This value is in good agreement with other values found in this and other laboratories (1,2).

C. Survival under Chronic Irradiation to Death.

The survival curve for animals given repeated exposures to 166 rad of neutron at intervals of 4-5 weeks is shown as the solid line of Figure 2, where fraction of the original number of animals surviving is plotted against duration of experiment. The survival curves for the groups of mice given X-ray exposures of 280 and 420 rad each time the neutron radiation was given to the neutron group are shown as dashed

lines of Figure 2. In addition, one other X-ray control was obtained as follows: the rate of neutron exposure was calculated as average dose per week, and from among mice of the same strain given weekly X-ray doses in previous experiments, a set of mice was selected which had received a weekly dose most closely corresponding to the product (neutron weekly dose rate) x (neutron RBE for acute LD<sub>50</sub>). This product was equal to 88.5 rad/week, and the closest corresponding X-ray exposure was 93 rad/week. The survival curve is plotted as a dotted line on Figure 2.

The exposure data for these repeated exposure experiments are summarized in Table II. From Figure 2, it can be seen that the survival curve for the neutron-irradiated group of mice is clearly bracketed by the survival curves for the groups of mice given 420 and 280 rad of X-rays each time the neutron exposure was given, and lies close to the survival curve for mice given 93 rad/week of X-rays. Referring to column 5 of Table II, one would calculate that the RBE for repeated exposure under these conditions is less than  $\frac{104}{38.5}$ , or 2.7, greater than  $\frac{56.7}{38.5}$ , or 1.5, and close to  $\frac{93}{38.5}$ , or 2.4. Thus, it can be concluded that the RBE for repeated exposure to neutrons under the conditions of the present experiment is not significantly different from that found for a single exposure.

## DISCUSSION

The original question which led to the present investigation was whether irradiation with fast neutrons caused a significantly greater amount of residual injury than X- or  $\gamma$ -radiation, relative to doses producing various acute responses. On the basis of the measurement of non-recuperable injury produced by neutron irradiation, this question can be answered simply: no. The relative amount of non-recuperable injury produced by neutron irradiation, expressed as percent of the amount of conditioning irradiation, is not significantly different from the relative amount produced by X-ray. It is concluded that the RBE for residual radiation injury is the same as that for acute lethality.

The present results complement and reinforce those of Curtis (12) and Henshaw (6), who found that RBE for life span reduction in mice from single doses of fast neutron irradiation was not different from that for acute lethality. A similar conclusion was reached by Upton (13) in the case of thermal neutrons. Other studies by Baum, Davis, and Alpen (14) have shown that the RBE for residual injury to the hemopoietic system of dogs is not significantly different from the RBE for acute lethality. Thus, the notion that the neutron RBE for residual injury is generally greater than that for acute injury must be discarded.



It is appropriate at this point to examine the past evidence which led to the opinion that RBE for residual radiation injury was greater than that for acute radiation injury. Two independent studies by Henshaw (6) and Evans (7) were conducted in an almost identical manner and gave almost identical results. In both experiments, the gamma:neutron dose ratio for acute lethality was compared with the dose ratio giving equivalent survival under chronic or repeated exposure conditions lasting up to 6 months or longer. The gamma:neutron dose ratio for acute lethality was 8:1 or 9:1 (using an ordinary thimble chamber to measure both neutron and  $\gamma$ - or X-ray doses), and this ratio increased to between 20:1 and 35:1 for survival under repeated or chronic exposure conditions lasting 6 months or longer. Thus, the RBE for neutron irradiation was increased by a factor of between 2.5 and 4 when the time of exposure to the radiation was sufficiently extended. A similar conclusion is suggested by results reported by Mole (5), in which the value of RBE for death from chronic radiation was found to be 13. Unfortunately, Mole did not report a measured value of the RBE for acute lethality, but the values reported in other references (1-4), as well as the present study, suggest that the value for the acute lethality RBE should be between 1.7 and 4.5. Thus, Mole's value for the RBE of chronic exposure is between 3 and 7.5 times as high as a reasonable value for the RBE of a single, acutely-lethal dose.

It can be concluded, then, that chronic neutron irradiation can have a higher effectiveness in foreshortening survival of animals than measurements of acute lethality would indicate. The evidence of the present study, as well as of others in the past, affirms that neutron irradiation has no intrinsic capacity to cause greater residual injury than its RBE would indicate. The question of rate of recovery from single doses of neutron radiation was investigated by Melville (15), who concluded that the rate of recovery from single doses of neutron radiation was the same as that from single doses of X-ray. It follows that the accumulation of injury by animals exposed to chronic neutron irradiation does not proceed in the same manner as with exposure to X- or  $\gamma$ -radiation, and this difference is not explainable in terms of differences in recovery rates or fractions of residual injury.

On the basis of the data at hand, it is possible to make some estimate of the region of experimental procedure in which this anomaly is likely to influence the apparent value of the RBE. In the experimental results above, there was no change of RBE from the acute lethality value when the chronic radiation was given as a series of large doses at long (5 week) time intervals over a period of 5 months. In the experiments of Evans (7) there was no change of RBE when the chronic radiation was given as a series of daily exposures for 25 days, but the RBE did increase when the chronic radiation was given over a period of 6 months or longer. Similar results for protracted exposure

were reported by Henshaw (6). Finally, Mole reported studies of total accumulated injury (both residual and recoverable) from exposure to continuous neutron irradiation at 18 rad/week (16). The total injury was found to parallel that produced by exposure to 110 rad/week of  $\gamma$ -irradiation for a period of 20 weeks, but at 30 weeks the neutron injury was accumulating faster than the  $\gamma$ -injury. The author attributed the later divergence to experimental variation, but in the light of the other results reviewed here the divergence may be real. The value of RBE for the first 20 weeks was  $110/18$ , or 6, which is in the neighborhood of what value of RBE would be expected for acutely lethal responses with the type of neutron dosimetry employed. Again, it would be helpful if direct data were available.

The sum of results suggests that when chronic or repeated irradiation of mice is carried out over a period of 20 weeks or less, the injury caused by neutron irradiation will accrue at the same rate as will that caused by X- or  $\gamma$ -irradiation, and the RBE for the chronic neutron irradiation will be the same as that for a single dose. Beyond 20 weeks, the rate of injury accumulation from neutron irradiation will become greater than that from X- or  $\gamma$ -irradiation, and the RBE for the chronic neutron irradiation will become greater than that for a single dose.

#### SUMMARY

The non-recuperable injury caused by exposure of mice to fission-spectrum neutrons was found to be the same as that caused by exposure to X-ray (9-10% of the conditioning dose). On this basis, it was concluded that the relative biological effectiveness (RBE) of neutrons for causing permanent residual injury was the same as that for causing acutely lethal injury. In a study of the tolerance of mice to regularly repeated neutron irradiation, it was found that the RBE for tolerance to the repeated irradiation was the same as that for tolerance of a single acutely lethal dose. A survey of the literature on RBE for tolerance of repeated neutron irradiation indicates that when the time span of irradiation exceeds 5 months, the RBE for tolerance to chronic neutron irradiation increases above that for tolerance of acutely lethal injury.

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<p>Naval Radiological Defense Laboratory USNRDL-TR-550</p> <p>RESIDUAL INJURY CAUSED BY IRRADIATION WITH FAST NEUTRONS by J.S. Krebs and R.W. Brauer 28 February 1962 25 p. tables illus. 4 refs. UNCLASSIFIED</p> <p>The LD<sub>50</sub> of C<sub>3</sub>H female mice for fission-spectrum neutrons was found to be <math>274.5 \pm 4.2</math> rad. The previously reported* value in the same mice for 250 kvp X ray was 632 rad, giving an RBE of 2.30. Conditioning exposure of the mice to a total neutron dose of 482.3 <math>\pm 7.2</math> rad over an interval of 8-10 (over)</p> <ol style="list-style-type: none"> <li>1. Fast neutrons - Dosage determination.</li> <li>2. Fast neutrons - Biochemical effects.</li> <li>3. Radiation injuries - Analysis.</li> <li>4. Radiation tolerance - Measurement.</li> <li>5. X rays - Biochemical effects.</li> </ol> <p>I. Krebs, J.S. II. Brauer, R.W. III. Title. IV. NR005.08-1200. UNCLASSIFIED</p>	<p>Naval Radiological Defense Laboratory USNRDL-TR-550</p> <p>RESIDUAL INJURY CAUSED BY IRRADIATION WITH FAST NEUTRONS by J.S. Krebs and R.W. Brauer 28 February 1962 25 p. tables illus. 4 refs. UNCLASSIFIED</p> <p>The LD<sub>50</sub> of C<sub>3</sub>H female mice for fission-spectrum neutrons was found to be <math>274.5 \pm 4.2</math> rad. The previously reported* value in the same mice for 250 kvp X ray was 632 rad, giving an RBE of 2.30. Conditioning exposure of the mice to a total neutron dose of 482.3 <math>\pm 7.2</math> rad over an interval of 8-10 (over)</p> <ol style="list-style-type: none"> <li>1. Fast neutrons - Dosage determination.</li> <li>2. Fast neutrons - Biochemical effects.</li> <li>3. Radiation injuries - Analysis.</li> <li>4. Radiation tolerance - Measurement.</li> <li>5. X rays - Biochemical effects.</li> </ol> <p>I. Krebs, J.S. II. Brauer, R.W. III. Title. IV. NR005.08-1200. UNCLASSIFIED</p>
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